Supplemental Table 1. *Everolimus affects cell cycle in a genotype dependent fashion.*

hTERT-HME1 cells of the indicated genotype were incubated for 48 h with everolimus (0.5 µM), and the effect on cell cycle was analyzed by FACS. Upon treatment, only hTERT-HME1 *PIK3CA* KI clones significantly accumulated in the G0/G1-phase of the cell cycle. Accordingly, the proportions of cells in the S- and G2/M-phases decreased. Means of at least 4 independent experiments are shown. Significance by paired t test was taken at $p<0.01$.

	DMSO		Everolimus 0.5 µM		p
	Mean	SD	Mean	SD	
hTERT-HME1 WT					
G1	71.6	6.6	75.7	5.9	0.029
G2/M	14.2	2.2	14.2	4.6	0.979
S	14.7	3.9	10.1	1.7	0.047
G2+S	28.9	5.9	24.3	6.0	0.022
Sub-G1	1.0	0.3	0.9	0.4	0.543
KI PIK3CA E545K					
G1	67.5	5.0	78.5	1.2	**0.002**
G2/M	17.9	4.8	12.7	3.1	0.015
S	14.6	2.4	8.8	3.5	**0.002**
G2+S	32.5	5.0	21.5	1.2	**0.002**
Sub-G1	1.0	0.3	0.9	1.2	0.811
KI PIK3CA H1047R					
G1	73.0	3.6	88.7	4.7	**0.003**
G2/M	9.6	0.6	4.9	1.2	**0.003**
S	17.4	3.5	6.4	4.3	**0.008**
G2+S	27.0	3.6	11.3	4.7	**0.003**
Sub-G1	1.4	1.3	0.6	0.4	0.181
KI KRAS G13D					
G1	73.3	6.2	75.7	9.0	0.325
G2/M	13.6	1.4	13.1	3.7	0.668
S	13.0	5.4	11.2	5.5	0.204
G2+S	26.6	6.2	24.3	9.0	0.331
Sub-G1	0.5	0.4	0.9	0.4	0.338
DKI (KRAS G13D + PIK3CA H1047R)					
G1	70.9	2.8	77.5	8.6	0.216
G2/M	10.8	1.3	10.5	3.9	0.869
S	18.2	1.6	12.0	4.7	0.077
G2+S	29.1	2.8	22.5	8.6	0.216
Sub-G1	5.2	1.4	5.0	2.3	0.754

Supplemental Table 2.

Genetic alterations of *PIK3CA, KRAS* and *BRAF* in cancer cell lines displayed in Figure 1C. The status of PTEN expression is also shown.

	PIK3CA	PTEN	KRAS	BRAF
PANC-1			G12D	
HT29	P449T			V600E
HCT116	H1047R		G13D	
DLD-1	E545K		G13D	
PC-3		loss		
U87-MG		loss		
ME-180	E545K			
BT474	K111N			
SK-OV-3	H1047R			
MCF7	E545K			
NIH:OVCAR-3	ampl			

Supplemental Table 3.

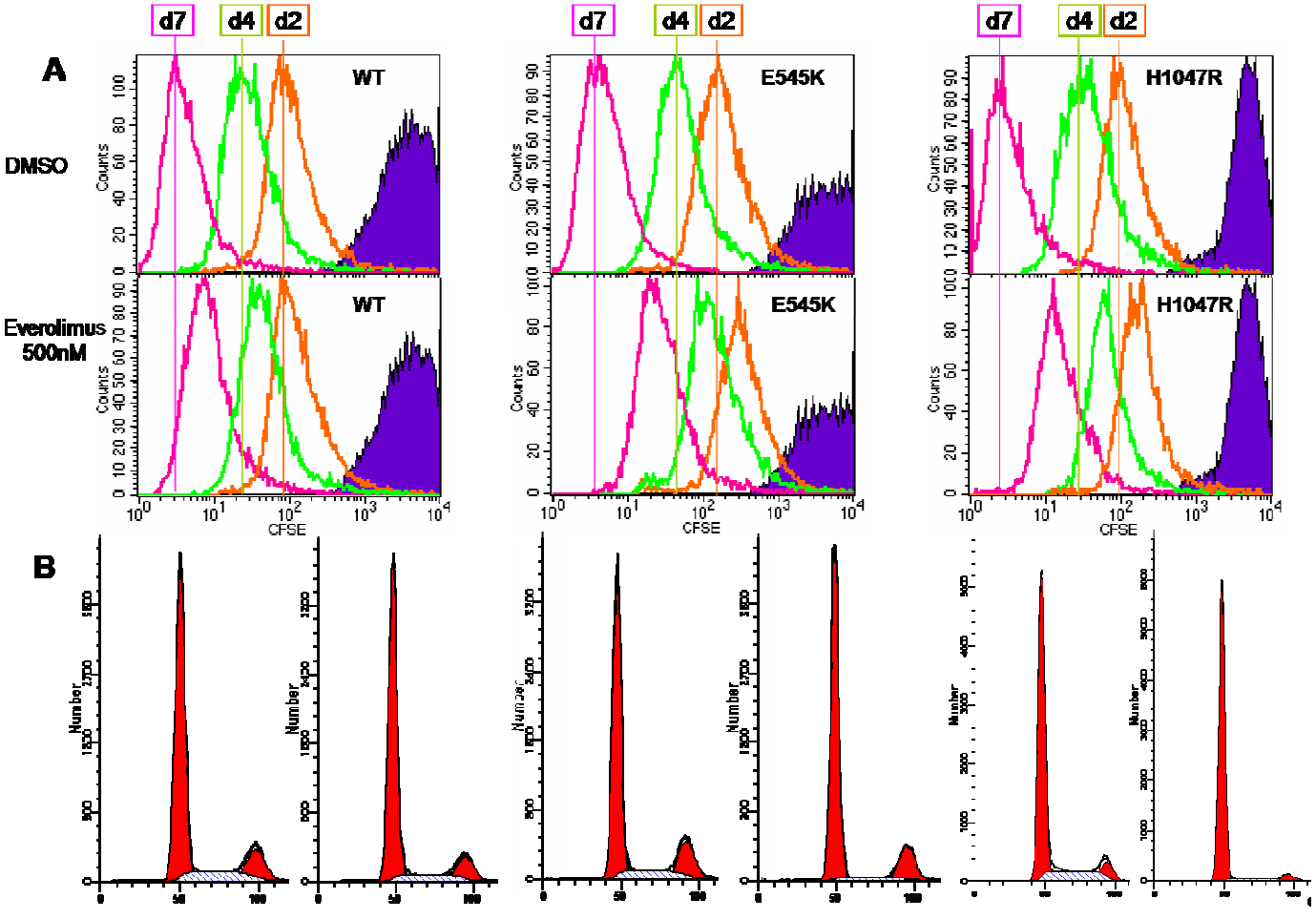
Genetic mutations of *PIK3CA* and *KRAS* in the isogenic cell lines employed in this study.

CELL LINES		MUTATIONS			
		PIK3CA		KRAS	
hTERT-HME1	WT	WT		WT	
	KI PIK3CA	H1047R		WT	
	KI PIK3CA	E545K		WT	
	KI KRAS	WT		G13D	
	DKI	H1047R		G13D	
MCF10A	WT	WT		WT	
	KI PIK3CA	H1047R		WT	
HCT116		WT	H1047R	WT	G13D
	WT	+	+	+	+
	HK2-6	+	+	-	+
	HKe-3	+	+	+	-
	HKh-2	+	+	+	-
	-/H1047R	-	+	+	+
	WT/-	+	-	+	+

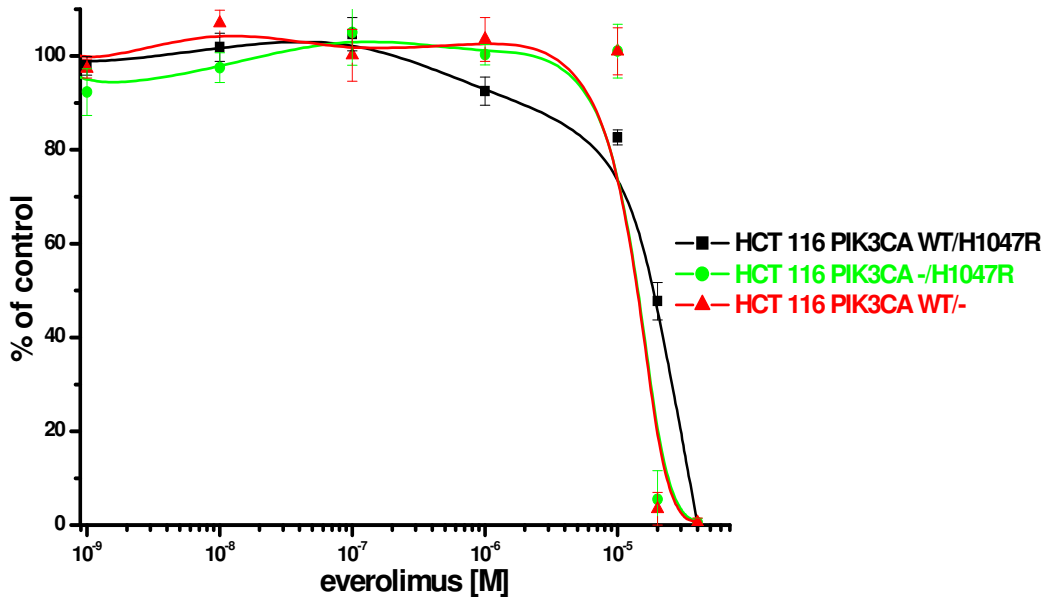
Supplemental Table 4.

Primers used for the amplification of the homology arms of the *PIK3CA* targeting vectors. The position of the mutated residues and the source of genomic DNA used for the PCR amplification are also indicated.

PIK3CA	Homology	gDNA source	Primers (F= forward; R=reverse)	Restriction sites
E545K	5'	hTERT-RPE1	F ttatttGATATCGCGGCCGCaggcttgcagtgttttctcc	Eco RV, Not I
			R ctggatGATATCatgatttacagaaaaagcaa	Eco RV
	3'	ME180	F tctgtaACTAGTctgtgaatccagaggggaaa	Spe I
			R gcacagACTAGTtggcaaagaacacaaaagga	Spe I
H1047R	5'	HCT116	F ggtttcGAATTCGCGGCCGCgctggtcttgaactcccaa	Eco RI, NotI
			R ttggagGAATTCatgttaataccttcaggtctttgc	Eco RI
	3'	HCT116	F aggtatTCTAGAcatttgctccaaactgacca	Xba I
			R tgtccaTCTAGA*ataacttcgtataatgtatgctatacgaagttat*GTGACTGCTTCCAAAACTGC	Xba I, loxP



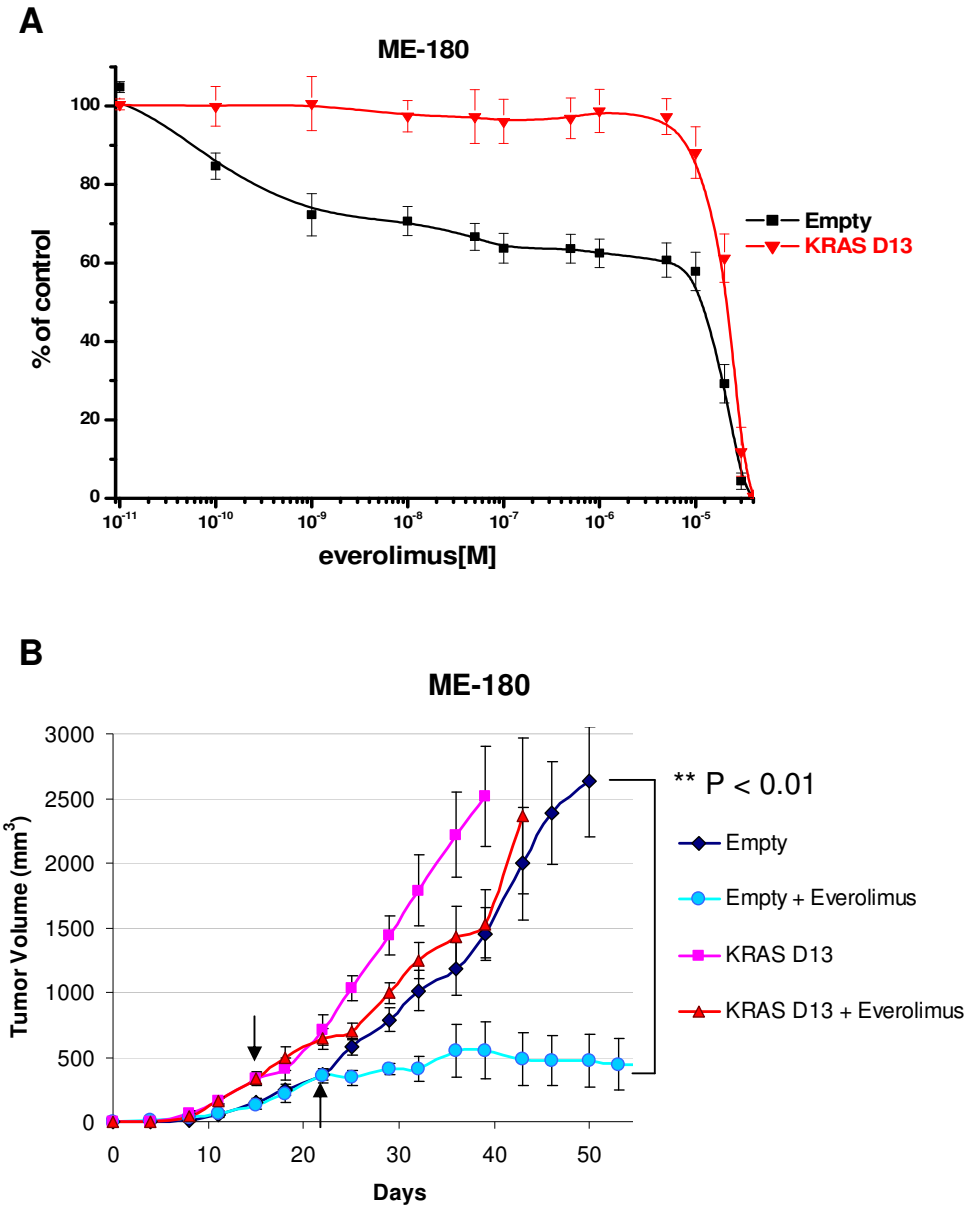
Supplemental Figure 1. *Everolimus affects cell cycle in a genotype-dependent fashion.* (**A**) CFSE-labeled cells were analyzed by flow cytometry at the indicated time-points. The maximum fluorescence intensity for all samples was recorded at day 0 (depicted in blue). Decrease of fluorescence intensity is proportional to the number of cell divisions. hTERT-HME1 WT, *PIK3CA* E545K and H1047R KI cells showed a similar pattern of cell doublings in absence of treatment. Exposure to everolimus 500 nM for 7 days resulted in decreased cell proliferation rate in all genotypes, with the effect being particularly evident in *PIK3CA* H1047R, less pronounced in *PIK3CA* E545K and only minimal in WT cells. (**B**) Cells of the indicated genotype were incubated with everolimus 500 nM for 48 h, after which cell cycle was analyzed by flow cytometry. No increase of the subG1 apoptotic fraction of cells was observed upon treatment. Representative data from 3 independent experiments are shown.



Supplemental Figure 2.

Effect of everolimus on HCT 116 cells and their PIK3CA WT or mutant derivative clones.

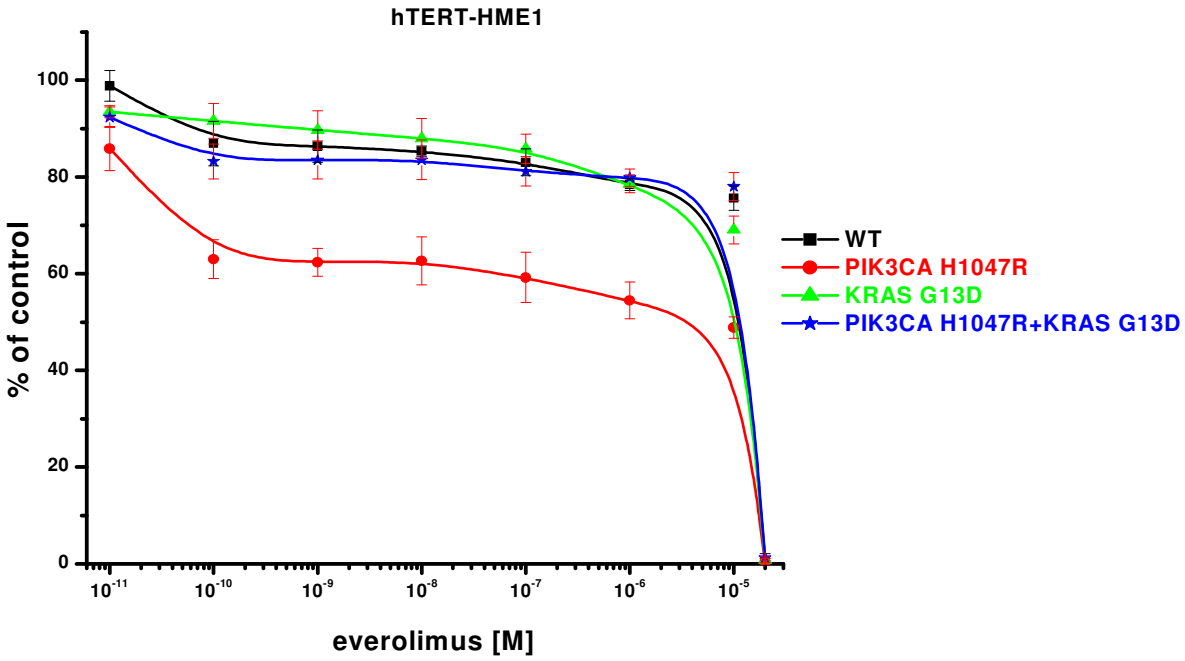
After 96 hours' treatment with everolimus, HCT 116-derived cells that are knock-out for the mutated 1047R allele of *PIK3CA* (WT/-, depicted in red) displayed similar response as either their parental cells (WT/H1047R, in black) or a clone retaining only the *PIK3CA* mutated allele (-/H1047R, indicated in green).



Supplemental Figure 3.

Oncogenic KRAS D13 confers resistance to everolimus in ME-180 cells.

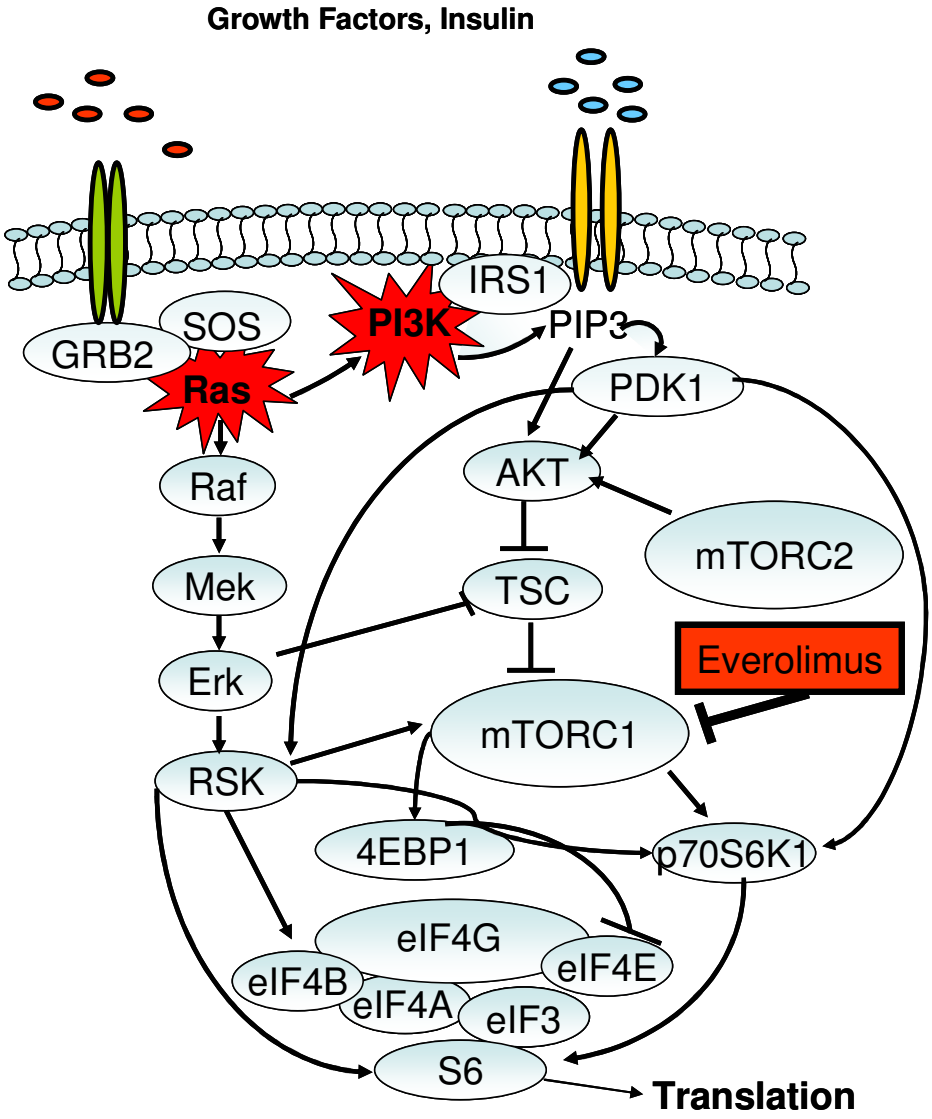
(**A**) Effects of everolimus treatment (72 hours) on proliferation of endometrial ME-180 cells infected with control or *KRAS D13* lentivirus. (**B**) NOD-SCID mice were inoculated with ME-180 cells (5 x 10^6) infected with empty or KRAS D13 lentiviral vectors; once tumors were established, animals were administered everolimus at 7.5 mg/kg three times a week. The arrows indicate the timepoint at which drug treatment was started. Results are shown as mean ± SEM.



Supplemental Figure 4.

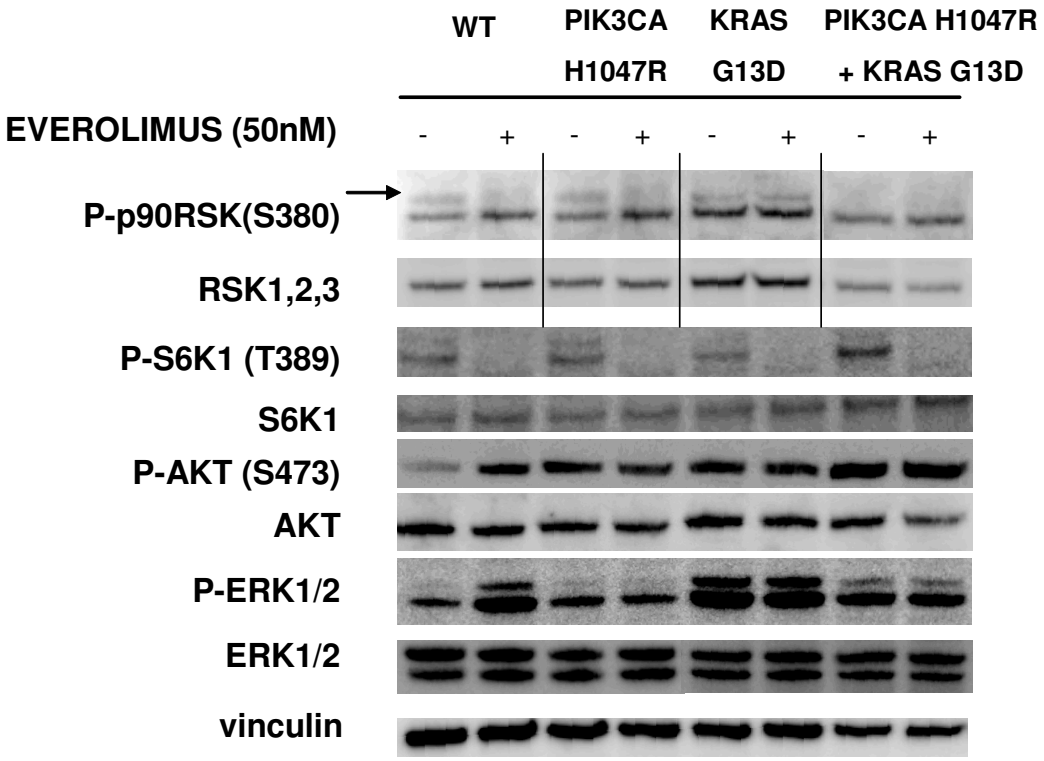
Effect of everolimus on hTERT-HME1 cells and their mutant derivative clones.

The effect of everolimus treatment on cellular proliferation was assessed for hTERT-HME1 cells and their isogenic clones carrying the indicated PIK3CA and/or KRAS mutations. The average cell number was measured by determining ATP content in three replicate wells. Results are normalized to growth of cells treated with DMSO and are represented as mean ±SD of at least three independent experiments.



Supplemental Figure 5.

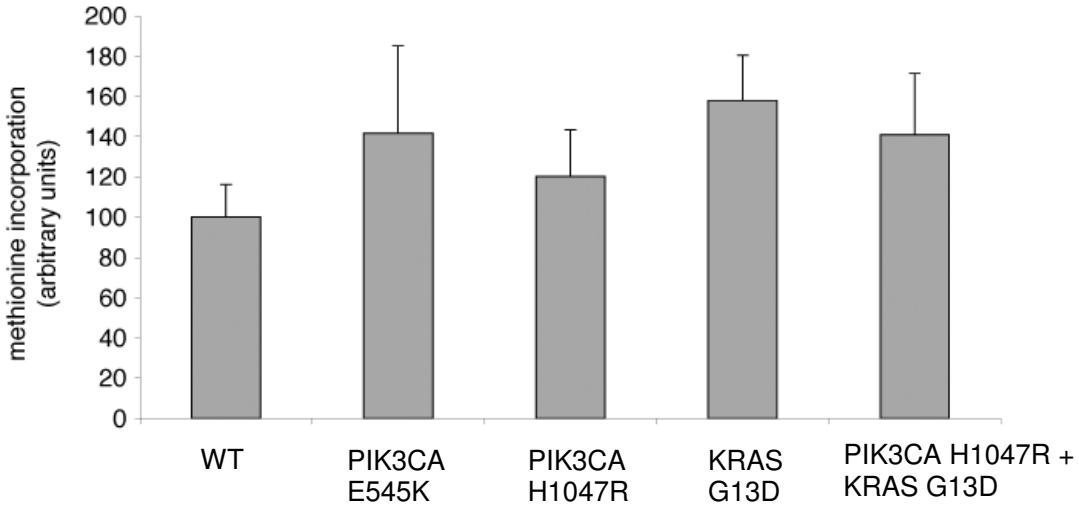
Schematic representation of the signaling pathways studied.



Supplemental Figure 6.

Effect of everolimus on AKT and MAPK signaling in hTERT-HME1 cells and their mutant derivative clones.

hTERT-HME1 cells carrying the indicated alleles were serum starved overnight and treated for 30 minutes with everolimus (50 nM); the corresponding lysates were blotted with total RSK1,2,3, phospho p90-RSK (Ser380), total S6K1, phospho S6K1 (Thr 389), total AKT, phospho AKT (Ser473), total ERK1/2 and phospho-ERK1/2 antibodies.



Supplemental Figure 7.

KRAS or PIK3CA oncogenic mutations do not affect basal translation rates.

hTERT-HME1 cells of the indicated genotype were pulsed with ^{35}S-methionine for 45 minutes. Metabolic incorporation was measured in newly translated proteins. Data (mean ± SD) are normalized over total protein content.